Original article

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EVALUATION OF THE VALUE OF MODIFIED NITRITE TEST IN EARLY DETECTION OF URINARY TRACT INFECTION IN CHILDREN

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Objective - The study was conducted in order to evaluate the value of the modified nitrite test in the diagnosis of urinary tract infection (UTI) in children.

Patients and methods - The study included 300 children of both sexes, aged 1 month to 14 years who were examined due to suspicion of UTI at the Department of Paediatrics, University Clinical Center Tuzla in the period from August 1, 2006 to August 1, 2007. In relation to the age, the patients were divided into two age groups: from 1 month to 2.0 years and from 2.1 years to 14 years. These groups will further be referred to as the younger and older groups. A cross-sectional study was used to analyze the biochemical examination of urine using test strips (nitrite and modified nitrite test) and the urine culture results.

Results - The described modification of the nitrite test significantly improved its sensitivity (88%) ($p=0.001$), NPV (96%) ($p<0.0001$), LR (-) (0.1) and it is more reliable than the nitrite test to exclude the existence of a UTI. The harmony between nitrite and modified nitrite ($k=0.66$, 95% CI: 0.56, 0.76, $p<0.001$) and between the modified nitrite test and the urine culture is good ($k=0.79$, 95% CI: 0.72, 0.87, $p<0.001$). Through reliability analysis of the modified nitrite test according to the age groups, we found that in the younger age group, after the modification of the nitrite test, its sensitivity was improved (83%), although not significantly ($p=0.153$), the NPV (94%) ($p<0.0001$) and LR (-) (0.2) was also improved. The harmony between the nitrite and modified nitrite test was moderate ($k=0.59$, 95% CI: 0.43, 0.75; $p<0.001$), and between the modified nitrite test and urine culture it was good ($k=0.79$, 95% CI: 0.61, 0.87; $p<0.001$). In the older age group, after the modification its sensitivity was significantly improved (93%) ($p=0.001$), NPV (98%) ($p<0.0001$); LR (-) (0.1). The harmony between the nitrite and the modified nitrite test was good ($k=0.72$, 95% CI: 0.60, 0.85; $p<0.001$), and between the modified nitrite test and the urine culture it was very good ($k=0.84$, 95% CI: 0.75, 0.94; $p<0.001$).

Conclusion - The value of the modified nitrite test is characterized by the improved reliability of the nitrite test to exclude the existence of a UTI, especially in older children, which is important for clinical practice.

Key words: Modified nitrite test ▪ Diagnostic value ▪ Urinary tract infection

Introduction

Urinary tract infection (UTI) is a term covering situations where a significant number of bacteria is found and proved by urine culture in non contaminated urine. Its significance is defined by the number of bacteria per unit volume of urine, and this number depends on the mode of sampling. In the case of a midstream urine specimen or urine collected in clean plastic bags, the significant number of bacteria is 100,000 per milliliter of urine (1). One waits for these results at least eighteen hours and very often rapid tests are used by clinicians in diagnostics in order to diagnose or to exclude the diagnosis of UTI, while waiting for the results of the urine culture (2). The most commonly used test is the test for the detection of nitrite in the urine (nitrite test). It is performed according to the manufacturer's procedure, and is based on the fact that gram negative bacteria from urine reduce nitrate into nitrite, which is not the case with gram positive bacteria. The test is primarily valuable for the diagnosis of a UTI when it is positive because the occurrence of nitrite in the fresh sample of urine indicates a UTI. It is best to use the first morning urine sample and to examine it within one hour after taking it, or refrigerate at + 4 °C, because at room temperature it breeds bacteria after only a short time , which can give false positive results.

Also, some drugs and compounds that contain nitrogen, as well as exposure of the test strips to air can give this result. A negative test result does not exclude a UTI because there are many causes of false-negative results. In pediatric practice, the most common cause is the short retention of urine in the bladder because for the conversion of nitrate to nitrite four hours incubation of urine in the bladder is necessary (1). In addition, there must be enough nitrates present in the urine, which serve as a substrate for the reaction and come from foods that are rich in vegetables, so the lack of nitrates in food due to diet or parenteral nutrition may also give false negative results. Taking large doses of vitamin C and more fruits, antibiotic treatment, or infections caused by bacteria that do not have the necessary reductase conversion of nitrate to nitrite can also give false negative results. Another test for the detection of nitrite in urine is a modified nitrite test (3). It is based on the fact that the reduction of nitrate to nitrite under the influence of bacteria that are found in the urine can occur in vitro. It is performed in case of negative test results of the nitrite test, with the addition of nitrate and incubation of the urine at 37 °C, and then again the presence of nitrite in the urine needs to be proved as in the previous procedure (3). Analysis of urine, in both cases, should be done from the same sample that is sent for the urine culture.

The study was undertaken with the aim of evaluating the value of the modified nitrite test in the diagnosis of UTI in children.

Patients and methods

The study included 300 children of both sexes, aged 1 month to 14 years who were examined due to suspicion of UTIs at the Department of Paediatrics, University Clinical Center Tuzla, in the period from August 1, 2006 to August 1, 2007. Samples were collected serially. A cross-sectional study was used to analyze the nitrite and modified nitrite test, compared to urine cullture results in terms of the existence of the UTI. In relation to age, the patients were divided into two age groups: from 1 month to 2 years and from 2.1 years to 14 years. These are referred to as the younger and older groups.

From each patient a midstream urine or urine sample was collected in plastic bags. The sample was immediately divided into three sterile containers. The urine sample from the first container (5 ml), was tested by the author of the research for the presence of nitrites in the Laboratory of the Clinic of Pediatric in Tuzla with gabOsticks® test strips (Nettetal, Germany), according to the manufacturer's procedure. The result is expre-

ssed in arbitrary units as positive or negative, and any degree of pink coloration was considered a positive result.

In the case of negative nitrite test results, the author of the study performed a modified nitrite test on the second urine sample. In l ml of urine, 1 drop of sterile 1% $NaNO_3$ solution was added, the sample was kept 4 hours in a thermostat at 37 °C, and then the presence of nitrite in the urine was re-examined using test strips as in the previous procedure (3). Any degree of pink coloration was considered a positive result. All positive nitrite test results were added to these results.

The urine sample from the third container was sent to the Polyclinic for Laboratory Diagnostics, University Clinical Centre Tuzla, for bacteriological examination by the standard procedure. The urine culture was the reference for the diagnosis of UTI and assessed the reliability of the spontaneous and modified nitrite test. The urine culture was considered positive if the number of bacteria was ≥100 000 per milliliter of urine.

Statistical analysis

The research had the features of validation studies. The reliability of the diagnostic tests was assessed by their sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), likelihood ratio for a positive test result (LR+) and likelihood ratio for a negative test result (LR-). A test of proportions was used to test the statistical significance between samples. A paired proportions test was used due to the same samples. The difference between samples was considered significant if $p<0.05$. The Kappa test was used to compare the level of conformity between the methods. Data were analyzed using the statistical software Arcus Quick Stat (5).

Results

Table 1 shows the results and findings of the nitrite and modified nitrite test compared to urine culture results in 300 patients.

The frequency of positive urine culture results for all patients was 76/300 (25.0%). 43/76 (56.6%) had a positive nitrite test result, and 67/76 (88.2%) modified nitrite test result. 33/76 (43.4%) had negative nitrite test result, and 9/76 (11.8%) had a negative modified nitrite test result but they had positive urine culture.

In the younger group, the frequency of the positive urine culture results was 36/134 (26.9%). 17/36 (47.2%) had a positive nitrite test and 30/36 (83.3%) had a positive modi-

Table 1 Numerical results of the ratio of the nitrite and modified nitrite test and urine culture results in 300 patients

Nitrite test	Urine culture		Total	Modified Nitrite test	Urine culture		Total
	Positive	Negative			Positive	Negative	
Positive	43	4	47	Positive	67 (43*+24)	15 (4* + 11)	82
Negative	33	220	253	Negative	9 (33**−24)	209 (220**−11)	218
Total	76	224	300	Total	76	224	300

* Initial positive ** Initial negative

Table 2 Numerical ratio of the results of the nitrite test, modified nitrite test and urine culture in the younger age group

Nitritni test	Urine culture		Total	Modified Nitrite test	Urine culture		Total
	Positive	Negative			Positive	Negative	
Positive	17	2	19	Positive	30 (17*+13)	8 (2* + 6)	38
Negative	19	96	115	Negative	6 (19**−13)	90 (96**− 6)	96
Total	36	98	134	Total	36	98	134

* Initial positive ** Initial negative

Table 3 Numerical ratio of the results of the nitrite test, modified nitrite test and urine culture in the older age group

Nitrite test	Urine culture		Total	Modified Nitrite test	Urine culture		Total
	Positive	Negative			Positive	Negative	
Positive	26	2	28	Positive	37 (26*+11)	7 (2*+5)	44
Negative	14	124	138	Negative	3 (14**−11)	119 (124**−5)	122
Total	40	126	166	Total	40	126	166

*Initial positive; **Initial negative

Table 4 Evaluation of the reliability of the results of the nitrite and modified nitrite test compared to urine culture results and age group

Reliability	Age groups					
	Total		Young group		Old group	
	Nitrite test	Modified nitrite test	Nitrite test	Modified nitrite test	Nitrite test	Modified nitrite test
Sensitivity (%)	57	88	47	83	65	93
Specificity (%)	98	93	98	92	98	94
PPV(%)	91	82	89	79	93	84
NPV(%)	87	96	83	94	90	98
LR (+) (95%CI)	31.7 (12.3-82.6)	13.2 (8.1-21.6)	23.1 (6.3-87.0)	10.2 (5.3-20.1)	40.9 (11.4-151.1)	16,6 (8.3-34.2)
LR (-) (95%CI)	0.4 (0.3-0.6)	0.1 (0.0-0.2)	0.5 (0.4-0.7)	0.2 (0.1-0.3)	0.3 (0.2-0.5)	0.1 (0.0-0.2)

PPV = Positive predictive value; NPV = Negative predictive value; LR (+) = Likelihood ratio for a positive test; LR (-) = Likelihood ratio for a negative test; CI = Confidence interval

fied nitrite test. 19/36 (52.8%) with a positive urine culture had a negative nitrite test and 6/36 (16.6%) with a positive urine culture had a negative modified nitrite test.

Table 3 shows the numerical ratio of the results of the nitrite test, modified nitrite test and urine culture in the older age group.

In the older age group, the frequency of a positive urine culture result was 40/166 (24.1%). 26/40 (65.0%) had a positive nitrite test, 37/40 (92.5%) had a positive modified nitrite test. 14/40 (35.0%) with a positive urine culture had a negative nitrite test and 3/40 (7.5%) with a positive urine culture had a negative modified nitrite test.

Table 4 shows the indicators of the reliability of the nitrite test and modified nitrite test compared to the urine culture results in diagnosing UTI.

In the diagnosis of UTI in children, the nitrite test is characterized by high specificity (98%), PPV (91%) and excellent LR (+) (31.7) but lower sensitivity (57%), NPV (87%) and weak LR (-) (0.4). Its sensitivity was significantly improved by the described modification of the test (88%) ($p=0.001$), NPV (96%) ($p<0.0001$), LR (-) (0.1). The harmony between the nitrite and modified nitrite test is good ($k=0.66$, 95% CI: 0.56, 0.76; $p<0.001$), between the nitrite test and urine culture it is also good ($k=0.63$, 95% CI: 0.52, 0.73; $p<0.001$) and between the modified nitrite test and urine culture it is good as well ($k=0.79$, 95% CI: 0.72, 0.87; $p<0.001$).

With the help of reliability analysis of the nitrite test results in the diagnosis of UTI in the younger age group, we found that it has high specificity (98%), PPV (89%) and

excellent LR (+) (23.1), a lower sensitivity (47%), NPV (83%) and weak LR (-) (0.5). After the modification of the nitrite test, its sensitivity has improved (83%), although not significantly (p=0.153), NPV (94%) (p<0.0001) and LR (-) (0.2) also improved. The harmony between the nitrite and modified nitrite test is moderate (k=0.59, 95% CI: 0.43, 0.75, p<0.001), between the nitrite test and urine culture is also moderate (k=0.53, 95% CI: 0.36, 0.70; p<0.001), and between the modified nitrite test and urine culture it is good (k=0.79, 95% CI: 0.61, 0.87, p<0.001).

In the older age group, the nitrite test is characterized by high specificity (98%), PPV (93%), and excellent LR (+) (40.9), a lower sensitivity (66%), NPV (90%) and LR (-) (0.3). After the modification its sensitivity significantly improved (93%) (p=0.001), NPV (98%) (p<0.0001) and LR (-) (0.1). The harmony between the nitrite and modified nitrite test is good (k = 0.72, 95% CI: 0.60, 0.85; p<0.001), between the nitrite test and urine culture it is also good (k=0.70, 95% CI: 0.57, 0.84; p<0.001), and between the modified nitrite test and urine culture it is very good (k=0.84, 95% CI: 0.75, 0.94, p<0.001).

Discussion

Reliability evaluation of the nitrite test compared to the urine culture results in children aged 1 month to 14 years was used and found that the nitrite test has a small number of false positives and a higher number of false negative results, resulting in low sensitivity and high specificity. The LR (+) and LR (-) also indicate that a child with a positive test is likely to have UTI if the urine is not contaminated, while a negative test does not exclude infection. However, this test has its limitations because of the fact that for the reduction of nitrate to nitrite, urine needs to be in the bladder for at least 4 hours, and in addition, the urine should have enough nitrates to serve as a substrate for the reaction. These are the most common reasons for a large number of false negative results, especially in infants and young children who urinate frequently. We thought this could be a cause in our study as well, and we conducted a survey according to age groups.

Reliability evaluation of the nitrite test in patients of the younger age group was used to find that due to its specificity, PPV and LR (+), it is reliable enough to prove UTI (one needs to think of the contamination of urine, for example, if urine samples were obtained in bags, they can give false positive results). However, the low sensitivity and low LR (-) make it unreliable to exclude the existence of the UTI, which confirms our assumption.

In patients of the older age group, the nitrite test is characterized by high specificity, the highest PPV and the highest LR (+), and with the greatest likelihood, a positive test result indicates a UTI. Compared to the younger age group, this group has fewer false-negative results and consequently a higher sensitivity and better LR (-) but it is not sufficient to exclude the existence of the UTI. Also, these results were expected and consistent with our assumption that age increases the reliability of the results.

In a study, Couthard et al. (6) analyzed 203 urine samples from children and it was found that the nitrite test is highly specific (0.98), which makes it valuable in demonstrating UTIs, but its sensitivity is low (0.61) so, it is uncertain in excluding one. Sadika et al. (7) analyzed 132 urine samples in children under the age of 12, and found that the nitrite test has a low sensitivity of 38.2% and NPV of 40.0% and 88.4% higher specificity and PPV 87.2% which is explained by the fact that it takes at least 4 hours for pathogenic bacteria to reduce nitrate into nitrite. They consider that a negative test result does not exclude a UTI. Tahirovic and Pasic (3) analyzed 306

urine samples in children under the age of 14 and found that the nitrite test has a high specificity (99.6%) and PPV (94.1%), a low sensitivity (21.3%) and NPV (79.6%), which is consistent with our results. By systematically reviewing 23 studies, Whiting et al. (8) examined the reliability of the nitrite test in the diagnosis of UTI in children under the age of 5. They found a relatively high share of LR (+) (15.9) and relatively low LR (-) (0.5), and concluded that this test can be reliable in proving a UTI but it is uncertain in excluding it. Downs and the American Academy of Pediatrics (9), by a systematic review of 12 studies, investigated the reliability of the nitrite test in the diagnosis of UTI in children aged 2 months to 2 years and found that it has high specificity of 98% (range 90% to 100%) and lower sensitivity of 53% (range 16% to 82%). Their opinion is that it is more reliable in proving UTIs than in excluding them.

Analyzing our results and those of other authors, it can be said that the nitrite test is very good for a quick diagnosis of UTI regardless of age because, if it is not contaminated, a positive result suggests the existence of UTI. However, although a negative result reduces the likelihood of UTI, this test is not reliable to exclude it. This is important because false-negative results may affect the prolongation of medical treatment while awaiting the urine culture results.

In order to improve the reliability of negative results of the nitrite test in the diagnostics of UTI in children, we evaluated a modified nitrite test according to the study by Tahirovic and Pasic (3) and, in our study, we performed a modification of the nitrite test in accordance with this study. We found that a modified nitrite test has fewer false-negative results, which significantly enhances its sensitivity, NPV, LR (-) and it is more reliable than the nitrite test to exclude the existence of UTI.

In the younger age group, after the modification of the nitrite test, its sensitivity improved, though not significantly. NPV and LR (-) also improved and the modified nitrite test was more reliable than the nitrate test to exclude the existence of UTI in younger children as well.

In the older age group, after the modification of the nitrite test, its sensitivity improved, as did NPV and LR (-), the harmony with the urine culture was very good, and the modified nitrite test was reliable to exclude the existence of the UTI in older children. Similar results were reported by Tahirovic and Pasic (3) who, in their study, analyzed 306 urine samples in children under the age of 14, before and after modifications, according to the established procedure. The initial nitrite test showed high specificity (99.6%) and PPV (94.1%), a low sensitivity (21.3%) and NPV (79.6%). After the modification, its sensitivity (93%) significantly ($p<0.01$) improved and NPV (98%) also improved which could confirm the hypothesis that insufficient incubation of urine in the bladder and the lack of nitrates in food leads to low sensitivity. They found the high sensitivity of the modified nitrite test very important for the detection and NPV for the exclusion of UTI. Sleigh (10) performed a different modification. The author found that in children with positive urine culture 59 samples had a negative nitrite test. After modification (in the urine sample, 5% KNO_3 solution was added, it was incubated at 37 °C for 6 hours and then he showed the presence of nitrites in the urine every hour), he received positive test results after 4 hours in 52 (88%), and after 6 hours in 57 (97%) urine samples. Barbin et al. (11) in their study analyzed the nitrite test in relation to positive urine culture and found that the test was positive in 18/51 (35%) samples, and after 4 hours incubation at 37 ºC in 23/51 (45%) samples. After 10 urine samples with a negative nitrite test result had been isolated, modification was performed (5% KNO_3 solution was added, it

was incubated at 37 °C for 6 hours and then they showed the presence of nitrites in the urine) after which 8 samples were positive and it was considered that adding nitrate and incubation improve the test accuracy. In the literature, data on the reliability of the modified nitrite test in relation to age groups was not found.

The results of our survey show that false-negative nitrite test results can appear due to the insufficient incubation of urine in the bladder, as well as the lack of nitrates from food, resulting in the fact that the nitrite test is not reliable enough to exclude the existence of the UTI. Modification of the test increases the reliability of negative results, especially in older children, which is important in diagnosing UTIs. The advantages of the modified nitrite test are that the test is available in clinical practice, practical and can be used for rapid identification of UTI. Although the shortcomings in sensitivity NPV and LR (-) are as acceptable as the risk of unnecessary initiation of antibiotic treatment, for the final diagnosis, in addition to nitrite and modified nitrite test it is mandatory to undertake urine culture tests. This is supported by the claim that the test must have sensitivity above 92% and specificity of over 99% in order to have an advantage over urine culture (9).

Conclusion

The value of the modified nitrite test in the diagnosis of UTIs is characterized by good reliability to detect UTIs, and better reliability than the nitrite test in excluding the existence of a UTI, especially in older children. Due to its characteristics, it can be very useful in clinical practice. But for final confirmation of the diagnosis and pathogen identification it is necessary to ask for at least one urine culture test.

Authors' contributions:
Conception and design: SZ and HT; Acquisition, analysis and interpretation of data: SZ and GI; Drafting the article SZ and HB; Revising it critically for important intellectual content: SZ and HT.

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